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THE EFFECTS OF LIGHT INTENSITY ON THE GROWTH RATES OF GREEN ALGAE[1,2,3]

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Among the environmental factors affecting the growth rates of unicellular algae, light is frequently at an improper level. In many laboratory cultures used for physiological research the light intensity is too low to permit logarithmic growth. In nature the intensity is well above saturation and may be high enough to inhibit growth during much of the day. The intensities for saturation and inhibition depend on the suitability of other factors of the environment, e.g., temperature, CO_2 level, and nutrient supply. In attempting to predict the performance of an alga under a given set of conditions it is necessary to know its potential under optimum conditions. Such information is basic to the evaluation of physiological studies and to the design and operation of culture apparatus. This paper describes the growth responses of five green algae to different light intensities under comparable environmental conditions.

MATERIAL AND METHODS

The algae for these studies included: *Chlorella pyrenoidosa* from the collection of Dr. Van Niel, Code No. Z.2.2.1. BAD 12/11; *Chlorella vulgaris* from Dr. Pringsheim's collection, No. 211/8b, listed as the one isolated by R. Emerson and used in his work on iron deficiency and photosynthesis; *Scenedesmus obliquus*, No. WH-50, isolated from fresh waters in Woods Hole by R. W. Krauss in 1950; *Chlamydomonas reinhardti*, plus strain, obtained from Dr. G. M. Smith through Dr. Harold C. Bold; and *Chlorella pyrenoidosa*, high-temperature strain 7-11-05, isolated in Texas in 1951 (10).

The cultures were grown in a medium of the following composition (in grams per liter): KNO_3, 1.25; KH_2PO_4, 1.25; $MgSO_4 \cdot 7\,H_2O$, 1.00; $CaCl_2$, 0.0835; H_3BO_3, 0.1142; $FeSO_4 \cdot 7\,H_2O$, 0.0498; $ZnSO_4 \cdot 7\,H_2O$, 0.0882; $MnCl_2 \cdot 4\,H_2O$, 0.0144; MoO_3, 0.0071; $CuSO_4 \cdot 5\,H_2O$, 0.0157; $Co(NO_3)_2 \cdot 6\,H_2O$, 0.0049; ethylenediaminetetraacetic acid (as a chelating agent), 0.5. The pH of the medium was 6.8. Test tubes with inner dimensions of 16 × 150 mm were used as growth vessels and the 10 ml of culture medium which they contained was inoculated from stock cultures maintained on agar slants. The test tubes were fitted with cotton plugs, through which cotton-plugged bubbling tubes were passed to supply the cultures with a 4 % CO_2-in-air mixture. After inoculation the cultures were placed in a constant temperature water bath made of lucite.

Illumination was provided by two batteries of lamps placed on opposite sides of the bath. Light intensities up to 2,000 ft-c were obtained with two batteries of four, white, 40-watt Champion, fluorescent lamps in each. Intensities above 2,000 ft-c were obtained with General Electric, 300-watt, medium-flood lamps. Reduced intensities were achieved by placing wire screens between the light source and the water bath. Light intensities were measured by a recently calibrated Weston illumination meter with the photocell inside the bath at the level of the culture tubes. It is recognized that the actual light intensity received by any cell in the algal suspension may differ from that measured by the photocell. There are two reasons for this discrepancy. First, cultures receive light from both sides of the bath as well as from reflection and scattering of light from other points around the culture tube. This situation is constant during each of subsequent experiments as long as the technique remains unchanged. Nevertheless, data obtained in different laboratories with different sources, different geometry of illumination, and different culture vessels may not be directly comparable. Second, as the culture grows, mutual shading of the cells reduces the actual amount of light received by a particular cell. It has been assumed that this factor does not influence growth as long as measurements indicate that the culture is in the logarithmic phase. Frequent transfers to fresh medium are required to prevent a reduction in growth rate. Deviations of curves from the exponential may also be due to transition phenomena taking place after transfer of cells from agar slants to liquid media. Therefore more than one transfer to fresh liquid media and a

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score of cell generations were often required before the lag phase was passed and growth measurements on steady-state suspensions could be started.

To measure growth, the optical density of the algal suspension was determined periodically by a photoelectric colorimeter with a filter transmitting at 600 $m\mu$. In a given algal suspension optical density is proportional to the density of the algal population in terms of cell numbers or cell protoplasm per volume of the suspension. In using optical density as a measure of growth the assumption is made that as long as the culture is in the exponential phase of growth under steady state conditions the proportionality between optical density and the density of algal population remains constant.

Growth rate, k, is calculated from the integrated growth equation:

$$k = \log_2 \frac{O.D._1}{O.D._0} \times \frac{1}{t},$$

where $O.D._0$ and $O.D._1$ are optical densities at the beginning and end of the time interval, t. By using logarithms to the base 2, and the unit of time as one day, the growth constant, k, becomes equivalent to the number of doublings per day. At least five readings were taken during exponential growth in each experiment at a particular light intensity. Reproducibility of the rate for at least two subsequent experiments was required. The logarithmic growth rate for each light intensity was calculated as an average from two experiments, each experiment conducted in duplicate.

Results

Figures 1 and 2 give the growth rates of five algae plotted against light intensities up to 10,000 ft-c.

Each of the light intensity curves consists of two portions. Illumination up to 2,000 ft-c was provided by fluorescent lamps. Intensities above 2,000 ft-c were from incandescent lamps. The shapes of the curves might not be identical were there only one source of light throughout the intensity range. However, it is probable that the curves would not differ much. Myers (5, 6), using incandescent lamps, obtained a growth curve for a low-temperature strain of *Chlorella pyrenoidosa* similar to the curve reported here for *C. pyrenoidosa* using fluorescent lamps. The two portions of the curve fit rather well and provide a satisfactory continuation for each other.

A typical light intensity curve for growth resolves itself into three distinct portions: 1) a light dependent portion in which the growth rate rises with the increase in light intensity, 2) a light independent portion or plateau, and 3) a light dependent portion in which the rate declines with increase in light intensity. In all these portions the characteristics of the curve depend heavily on the genetic make up of the organism (fig 1), and on external conditions other than the change in light intensity. Of the external conditions temperature is of primary importance (fig 2).

The light dependent portion of the curve may differ in its slope, in the degree of deviation from a straight line, and in the position of the point where it reaches light saturation. The differences in the slope indicate that the efficiency of utilization of incident light energy depends on the genetic make up of the organism and on external conditions.

The differences in the slope of the light intensity curves for growth, as one moves from lower to higher light intensity, were described by Bristol Roach (1) and observed by Phillips (8). They are also clearly indicated by Myers' data (5) though no reference to them was made. A similar phenomenon has also been observed for photosynthesis by Kok (2, 3, 4). The universal occurrence of this phenomenon is better demonstrated by plotting the lower portions of the curves on a larger scale (fig 3). It is more probable that the breaks, as they appear on the curves in figure 3, are due to discontinuity of measurements; the actual changes in the slopes of the curves are probably continuous and gradual.

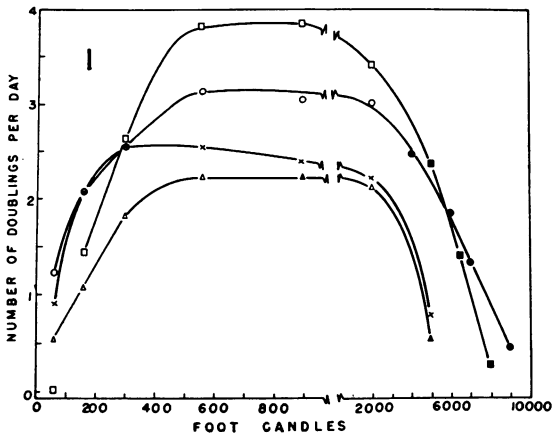
The steepness of the slope of a particular portion of the curve can be calculated in terms of the change in growth rate per 100 ft-c of change in light intensity. These data (α) for the lowest portion of each curve are compared in table I with those for the slopes of the same curves for the interval between 160 and 260 ft-c. Figures in the last column of table I indicate that the growth rates for the 2nd interval constituted only 19 to 58 % of those for the 1st interval. The lowest portions of the light intensity curves must actually have slopes steeper than those shown in figure 3 and the percentage values given in the last column in table I should be even lower. A zero point for growth cannot lie at the zero light intensity but should be moved up to a light intensity at which

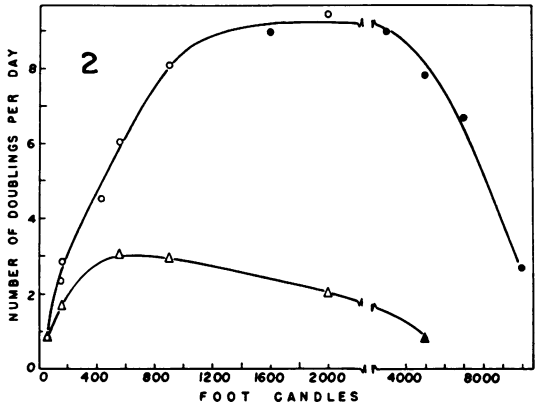
Table I

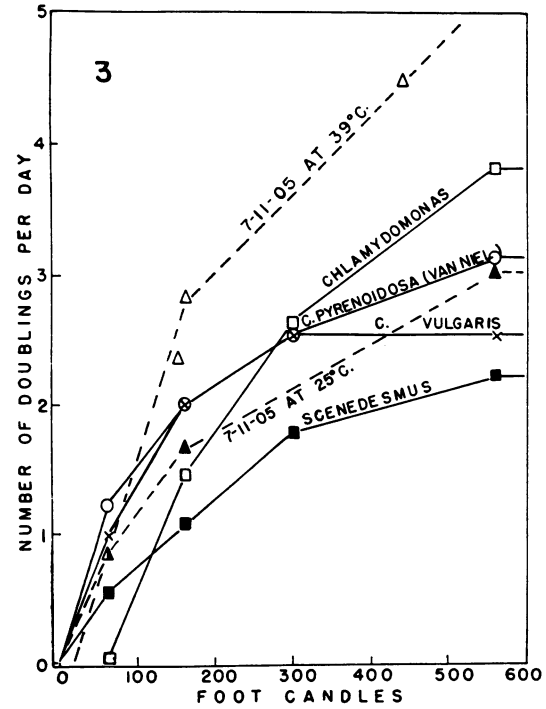
Increase in the Growth Rate (α) in Units of Log_2 per 100 ft-c for Six Green Algae

Organism	Temperature, °C	α units for the intervals of the curves: 0 to 100 ft-c	160 to 260 ft-c: Absolute	160 to 260 ft-c: Per cent
Chlorella pyrenoidosa, (van Niel)	25	2.00	.38	19
Chlorella pyrenoidosa, Emerson (from Myers, 5) *	25	1.20	.56	47
Chlorella vulgaris	25	1.64	.39	24
Scenedesmus obliquus, WH 50	25	.88	.50	57
Chlamydomonas reinhardti	25	1.43	.83	58
Chlorella pyrenoidosa, 7-11-05	25	1.36	.35	26
Chlorella pyrenoidosa, 7-11-05	39	1.70	.60	35

* Recalculated for a saturating light intensity of 400 ft-c according to the correction of Myers (6).







losses by respiration are just compensated by formative gains. For Chlamydomonas this compensation point for growth is above 60 ft-c; for other strains it lies at lower light intensities.

It can be concluded from table I that considered in terms of growth the maximum efficiency of utilization of incident energy, observed at the lowest light intensity, differed widely with various strains. It was more than twice as high in *C. pyrenoidosa* (van Niel) than in Scenedesmus. In *C. pyrenoidosa*, strain 7-11-05, it was higher at 39 ° than at 25° C.

A relatively high efficiency of utilization of incident energy at low light intensities is of great ecological importance. Because of this, an alga may be able to survive better in shaded or deep water. Efficiency must also be considered in predicting yields of mass cultures, since the density of algal populations often limits the light intensity received by the algal cells. It is obvious that any discussion on the utilization of light energy may be of little value unless the dependence of the " efficiency " of the species or strain on the environmental factors employed is clearly recognized.

Because of the gradual transition from the light-dependent to light-independent portion of an intensity curve, the lowest intensity which gives light saturation can only be approximated. For *Chlorella vulgaris* the data obtained in these experiments (table II) indicate that an increase in light intensity above 250 to 300 ft-c did not result in any increase in growth rate. For other strains at 25° C, the value was about 500 ft-c. This value is close to that given by Myers (6) and by Phillips and Myers (9) for the light-saturating intensity for the Emerson strain of *Chlorella pyrenoidosa*. Myers (6) estimated it to be in the neighborhood of 400 ft-c and Phillips and Myers (9), using different technique, found it at approximately 600 ft-c. It should be recognized, of course, that such determinations depend on the light source and the geometry of illumination. A characteristic feature of *Chlorella pyrenoidosa*, strain 7-11-05, is its higher requirement for light saturation at 39° C than at 25° C. Figure 2 shows that higher temperature will be of ad-

FIG. 1. The growth rates of four species of algae at 25° C measured at limiting, saturating, and inhibiting light intensities. The symbols are as follows: *Chlorella pyrenoidosa* (van Niel), circles; *Chlorella vulgaris*, crosses; *Scenedesmus obliquus*, triangles; and *Chlamydomonas reinhardti*, squares. Open symbols show growth under fluorescent light; closed symbols show growth under incandescent light.

FIG. 2. The growth rates of *Chlorella pyrenoidosa*, strain 7-11-05, measured at limiting, saturating, and inhibiting light intensities. Circles plot rates at 39° C; triangles plot rates at 25° C. Open symbols show growth under fluorescent light; closed symbols show growth under incandescent light.

FIG. 3. The growth rates of five strains of green algae measured at limiting light intensities. Data are those from figures 1 and 2 replotted on a larger scale.

TABLE II

GROWTH CHARACTERISTICS OF FIVE STRAINS OF GREEN ALGAE

ORGANISM	TEMPERATURE, ° C	SATURATION LIGHT INTENSITY, FT-C	GROWTH RATE AT HALF SATURATION	GROWTH RATE AT LIGHT SATURATION	LIGHT INTENSITY AT WHICH THE GROWTH RATE DECLINES TO ONE HALF OF ITS LIGHT-SATURATION VALUE
(1)	(2)	(3)	(4)	(5)	(6)
Chlorella pyrenoidosa (van Niel)	25	500	2.4	3.1	6500
Chlorella vulgaris	25	250	1.9	2.6	4500
Scenedesmus obliquus, WH 50	25	500	1.5	2.2	4500
Chlamydomonas reinhardti	25	500	2.2	3.8	6000
Chlorella pyrenoidosa, 7-11-05	25	500	2.3	3.0	3500
Chlorella pyrenoidosa, 7-11-05	39	1400	7.0	9.2	8500

vantage to this strain at any light intensity. An overall comparison of strains in the light-dependent portions of the light intensity curves is provided by the growth rates at half saturation (column 4 in table II). For *C. pyrenoidosa* 7-11-05 at 25° C the half saturating growth rate was practically the same as for *C. pyrenoidosa* (van Niel). At 39° C the growth rate for 7-11-05 was three times higher than at 25° C. The lowest rate at half saturating intensity was for Scenedesmus.

The maximum growth rate exhibited by cells at light saturation differed widely with the strain (column 5 in table II). At 25° C it was almost twice as high for Chlamydomonas as for Scenedesmus. The effect of temperature was more pronounced. For the *Chlorella pyrenoidosa*, strain 7-11-05 at 39° C the growth rate was more than three times higher than at 25° C.

The injurious effects of high light intensities upon algal growth have long been known but no detailed description of the phenomenon is available. As seen from figure 1 the deleterious effects of light at 25° C began at an unexpectedly low light intensity. The light-independent plateaus for the van Niel strain of *C. pyrenoidosa* and Scenedesmus extended from 500 to about 2,000 ft-c. It was shorter for Chlamydomonas, stretching from 500 to not more than 1,000 ft-c. A short plateau from 250 or 300 to not more than 600 ft-c is characteristic of *C. vulgaris*. The low light-saturating intensity and the low intensity injurious to growth of this strain are features characteristic of shade plants.

The description of an organism as a shade plant can be only relative. *C. pyrenoidosa* strain 7-11-05 at 25° C is very sensitive to increasing light intensities (fig 2). Injurious effects began at about 1,000 ft-c. At 39° C this strain showed no deleterious effects of high light intensity up to and probably above 3,000 ft-c. Because of the uncertainty as to the point where the light-independent portion (plateau) of the light intensity curve starts to bend, the light intensity at which the growth rate is depressed to one half of its maximum value is given in column 6 of the table II. The differences between the strains and the importance of the temperature factor are clearly evident.

Working with another strain of *Chlorella pyrenoidosa* Phillips and Myers (9) did not detect injurious effects of light intensities on growth up to 6,000 ft-c. However, differences in technique may provide an explanation for the discrepancy between their data and ours. Their experiments were performed in a flat growth chamber 1.0 cm thick with unilateral illumination. The density of algal populations was kept at a constant and much higher level than the initial optical density in the experiments reported here. Consequently mutual shading of cells may also have played a part in the absence of inhibition at their highest intensity. In the present study the mutual shading of cells was reduced to a minimum.

SUMMARY

The growth rates of *Chlorella pyrenoidosa* (van Niel), *Chlorella pyrenoidosa*, 7-11-05, *Chlorella vulgaris*, *Scenedesmus obliquus*, and *Chlamydomonas reinhardti* were determined under light intensities up to 10,000 ft-c. The intensities at which saturation was developed were shown to vary from 250 ft-c for *Chlorella vulgaris* to 500 ft-c for the other species at 25° C. At 39° C *Chlorella pyrenoidosa*, 7-11-05, was light dependent at intensities below 1,400 ft-c. Inhibition from high light intensities at 25° C began at 600 ft-c for *Chlorella vulgaris* but not until 2,000 ft-c for *Chlorella pyrenoidosa* (van Niel). The other strains were intermediate. *Chlorella pyrenoidosa* 7-11-05 was inhibited above 1,000 ft-c when grown at 25° C but not below 3,000 ft-c at 39° C. The validity of comparisons of these data with those of earlier workers was discussed, particularly with regard to differences in source and geometry of illumination. The growth rates under increasing light intensities were shown to be a function of the genetic make up of the species and temperature.

The change in slopes of the growth rate curves in the light dependent ranges were described for each

species. It appears that a decrease in the slope of the light intensity curve is a characteristic common to these algae and others that have been studied previously.

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THE OXYGEN AFFINITY OF A FLAVIN OXIDASE INVOLVED IN THE RESPIRATION OF STREPTOCOCCUS FAECALIS[1,2]

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The characterization of the oxidases involved in cell respiration has been based largely on experiments with enzyme inhibitors. In addition, it is possible to characterize an oxidase by its affinity for oxygen, which may be determined by measuring the oxygen consumption as a function of oxygen concentration. This information is especially valuable in those cases where the tissue respiration is insensitive to such classical inhibitors as cyanide and carbon monoxide (see 1, 4, 6). Although it has often been assumed that this "insensitive" respiration is mediated by oxidases which are flavoproteins, recent studies (15, 17) have shown that these same tissues may exhibit a high oxygen affinity, much greater than that exhibited by most isolated flavin oxidases (12). This poses the following question: are there some other flavin oxidases with a relatively high oxygen affinity which could account for this type of respiration? To answer this, a study was made with *Streptococcus faecalis* (B33A) whose respiration is known to be mediated by a flavoprotein oxidase (2, 3). The great advantage of this particular organism is that it does not contain any cytochromes (13), which might complicate the interpretation of the results.

The culture methods employed were those described previously by Seeley and Vandemark (11). The cells were harvested in a centrifuge, resuspended in distilled water, and their respiratory rates determined, in the presence of glucose, by standard manometric techniques at 37.5° C (16). To test the effect of cyanide on respiration Robbie's $Ca(CN)_2$—$Ca(OH)_2$ mixtures were added to the center well (10). Cell-free extracts were obtained by grinding freshly harvested cells with alumina (A-301, Alcoa) in phosphate buffer (0.05 M, pH 7). The slurry was centrifuged at 2,000 × G for 30 minutes in the cold and the supernatant fraction, referred to as the enzyme solution, was used in the spectrophotometric assays. The oxidation of reduced diphosphopyridine nucleotide (DPNH) was followed at 340 mμ in a Beckmann DU spectrophotometer at room temperature, using cuvettes with a 1 cm light path. The effects of oxygen concentration were determined in vaccine-stoppered cuvettes which had been flushed 10 minutes with the appropriate gas mixtures. To obtain complete anaerobiosis, the special cell of Lazarow and Cooperstein was employed (8). Optical density units were converted to DPNH concentration by assuming a molecular extinction coefficient of 6.22×10^6 sq cm/mole (5). Protein was determined by the method of Stadtman et al (14).

In preliminary experiments, the well known fact that the respiration of *S. faecalis* is not inhibited by cyanide (9, 11) was confirmed using 0.001 M HCN. Following this, the effect of oxygen concentration on the oxygen consumption of whole cells was determined manometrically. The respiratory rates in vessels gassed with 5, 20 and 100 % O_2 showed only slight differences, whether the vessels were gassed initially or after a period in air. The rate in 5 % O_2 was not

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